SANITATION OF REVERSE OSMOSIS/ULTRAFILTRATION EQUIPMENT

F. E. McDonough and R. E. Hargrove
Dairy Products Laboratory
Eastern Marketing and Nutrition Research Division
Agricultural Research Service, U. S. Department of Agriculture
Washington, D. C. 20250

Abstract

Data are presented from studies relating to cleaning and sanitizing reverse osmosis/ultrafiltration equipment used to concentrate and fractionate cheese whey. Current equipment design makes cleaned-in-place (C.I.P.) systems mandatory since modules cannot be completely drained or disassembled. In addition, many presently accepted sanitizing methods cannot be used without harming the delicate membranes. More than 50 different chemical agents, or combinations, currently approved by the Food and Drug Administration, were screened. Grossly contaminated reverse osmosis/ultrafiltration equipment was readily sanitized with several agents, including iodophors (10 ppm available iodine), sodium metabisulfite (0.2%), diethylpyrocarbonate (0.05%), zephirin chloride (0.006%), and calcium hypochlorite (10 ppm available chlorine). Merits of these agents are discussed. The sanitizer of choice would depend on type and design of equipment, operation of the plant, time allotted for cleaning, and degree of control desired. For best results, complete flooding of test equipment was necessary, indicating that sterilization cannot be assured unless modules are mounted in a vertical position.

Much of the current food technology research is aimed toward developing systems which will not only solve pollution control problems but also recover valuable by-products. Of the many processes under consideration, reverse osmosis (RO) and ultrafiltration (UF) are the most significant. Although the two terms are often used synonymously, they differ in that RO is the term applied to separation of low molecular weight solutes (salts, sugars) from their solvents while UF is the term applied to separation of high molecular weight solutes (proteins, polymers) and colloidally dispersed substances from their solvents. The two processes can provide effective pollution control of processing wastes and in many instances can lead to profitable by-product recovery. Development work employing RO/UF during the past few years has involved fruit juices *(10)*, egg whites *(6)*, maple sap *(12)*, and cheese whey (*7, 8, 9*). Uses for RO and UF appear to be unlimited and as commercial use looms closer, attention has been shifted to sanitation.

Problems of sanitizing RO/UF equipment are difficult, since current equipment designs and the type of modular material used preclude use of many accepted sanitizing methods. Until new equipment is designed with sanitary fittings and more attention given to CIP sanitation, currently available sanitizers and procedures will have to be adapted to provide an acceptable level of sanitation. In addition to the usual requirements for any sanitizer regarding acceptability for food use, viz., efficacy against molds, yeast, and bacteria; lack of flavor residue; economy and ease of use; a sanitizer for RO/UF equipment must have no short or long term effects on the delicate membrane. In addition, both sides of the membrane and all other exposed surfaces must be accessible.

Some of the problems encountered during attempts to sanitize RO equipment used for concentration of maple sap have been described by Kissinger *(2)* and Kissinger and Willits *(3)*. Fenton-May et al. *(1)* also discussed parameters which must be considered in the design of a complete sanitary system. This paper reports on studies designed to find sanitizing agents for RO/UF equipment used to concentrate and fractionate cheese whey.

Materials and Methods

Equipment

Initial tests were made using individual tubular modules manufactured by Calgon-Havens Systems, San Diego, California.[1] Each module contained 18 fiberglass support tubes, 1/2 inch ID by 8 ft long, connected in series with plastic "U" turnarounds. Each tube was lined with a layer of cellulose acetate membrane and the bundle of 18 tubes was surrounded with a plastic shroud that provided protection and collected the permeate (liquid passing through the membrane), enabling it to flow through openings at the ends. Membranes designed for both RO and UF were utilized. The RO membranes, designated 3A, were moderately tight with a NaCl rejection rate of 75%. The UF membranes, type 215, were porous and rejected only solutes with a molecular weight larger than 15,000. Feed material was pumped into the single modules with a small centrifugal pump. Final tests were made using a complete pilot-plant unit containing 8 modules hooked in two parrallel series of four each. The modules were

[1]Mention of brand or firm names does not constitute an endorsement by the Department of Agriculture over others of a similar nature not mentioned.

mounted on a frame along with a Moyno screw pump (9P3S-SQ) designed to give pressures of up to 900 psi, and all necessary built-in valves and gauges.

Microorganisms

During periods of shutdown, samples were frequently collected from the unit for microbiological study. The predominant microorganisms isolated were *Escherichia coli, Pseudomonas* spp., *Flavobacterium* spp., *Saccharomyces fragilis,* and an unidentified mold. These isolates were maintained by subculturing in sterile dilute whey. They were used to determine germicidal properties of test chemicals and to contaminate the unit prior to sanitation tests. Total counts were determined on trypticase soy agar incubated 3 days at 30 C. Violet red bile agar, incubated at 37 C for 24 hr, was used to isolate *E. coli* and related types. *Pseudomonas* spp. and psychrophilic types were isolated from trypticase soy agar incubated at 5 C for 7 to 10 days. Malt agar, pH 4.5, incubated for 3 to 5 days at 22 C, was used to determine yeast and mold counts.

Preliminary screening procedure

Dilutions of test chemicals were added aseptically to flasks containing 1/100 dilutions of sterile sweet whey in distilled water. Flasks of whey and germicide were seeded with a 1% inoculum from 2- to 5-days old whey cultures of the test organism and incubated at 25 C. Samples were plated immediately and at 4, 10, 24, and 48 hr intervals. Those agents showing most promise were then tested, first in a single module and later in the complete pilot plant unit.

Test procedure

The general procedure for determination of the effectiveness of various sanitizers on the RO/UF equipment was as follows. After each use of the equipment for concentration and fractionation of whey, the unit was thoroughly flushed with tap water to remove as much whey residue as possible. This point was determined using a Myron L Dissolved Solids Meter to compare the water before and after passing through the unit. The minimum reading obtained was about 35 ppm of dissolved solids. Gross contamination of the modules was insured prior to each test by circulating whey-grown cultures of the test organisms through the equipment for 15 min. The equipment was then flooded by pumping dilutions of sanitizer through the unit for 15 to 20 min using several variations as follows: *(a)* sanitizer was pumped through the concentrate side only with modules in a horizontal position, *(b)* sanitizer was pumped through the concentrate side only with modules in a vertical position, *(c)* sanitizer was pumped through both the concentrate and permeate sides with the modules in a horizontal position, and, and *(d)* sanitizer was pumped through both the concentrate and permeate sides with the modules in a vertical position. When in a vertical position, the permeate and concentrate could exit only at the top. Each of these variations was tested using both the 3A and the 215 type membranes. Following each variation above, the equipment was shut down with the sanitizing solution remaining in the unit for periods varying from 3 to 48 hr. After each test period, the sanitizing solution was flushed out with and replaced by sterile water. Samples of water were removed immediately and periodically for an additional 48 hr and plated as a check on sterility.

Effect of prolonged contact between the sanitizing agent and the membrane was determined by completely flooding both the concentrate and permeate sides of the modules with the maximum recommended strengths of sanitizing solutions and storing for a period of 6 to 7 weeks. After storage, sanitizers were flushed out with tap water and permeability and rejection properties of membranes were compared to original values.

TABLE 1. EFFECT OF SANITATION ON TOTAL BACTERIAL COUNT OF STERILE FLUSH WATER HELD IN REVERSE OSMOSIS UNIT

Procedure	Holding time	Permeate side	Concentrate side	Water condition
	(hr)	———(No./ml)———		
	0	2×10^1	41×10^1	Clear - odor free
	24	50×10^3	40×10^5	Clear - unclean
A[a]	48	31×10^6	11×10^7	Cloudy - unclean
	96	21×10^7	36×10^7	Turbid - putrid
	0	<1	<1	Clear - odor free
	24	<1	<1	Clear - odor free
B[b]	48	<1	<1	Clear - odor free
	96	<1	<1	Clear - odor free

[a]Unit flushed 30 min with tap water, followed by 15 min flush with sterile water; counts were made on water in unit at periodic intervals.

[b]Unit flushed 30 min with tap water, sanitized 5 hr with 24 ppm iodophor, and flushed with sterile water; counts as in (a).

RESULTS AND DISCUSSION

The inability to maintain low bacterial populations in unsanitized RO equipment is shown in Table 1. Procedure "A" shows that, although a tap water flush did a good job of removing microorganisms, subsequent growth rapidly produced foul odors and unsanitary conditions. This demonstrated that flushing alone does not remove all traces of organic matter which can support microbial growth. The unit had been flushed until no further solids could be removed, as indicated by a 35 ppm reading on the water both entering and exiting from the unit—yet growth occurred. In contrast, sterilization by procedure "B" resulted in sanitary conditions with no evidence of growth in the sterile water after 4 days. A comparison of dissolved solids in the water from procedure "B" at 0 and 96 hr showed an increase from 35 ppm to 170 ppm, yet no microbial growth was detected. The increase was apparently due to leaching of traces of organic matter from the membrane into the water and is thus good evidence of the need for microbial control.

The more than 50 different chemical agents or combinations selected for screening were acceptable from a public health standpoint. All are currently included in the Food and Drug Administration code of Federal Regulations, either on the GRAS (generally regarded as safe) list or under special regulation categories. Hydrogen peroxide was not among those tested because strong oxidizing agents are known to be harmful to the membranes.

Table 2 lists some of the more lethal combinations in the optimum concentration as determined in the screening procedure using pure cultures in dilute water.

Table 2. Germicidal Effects of Sanitizing Agents in Dilute Whey[a]

Sanitizing agent	Conc. (%)	pH[c]	Microbial counts/ml[b]											
			Escherichia coli (hr)				*Pseudomonas* spp. (hr)				*Saccharomyces fragilis* (hr)			
			0	4	24	48	0	4	24	48	0	4	24	48
None	—	—	10^6	10^6	10^7	10^8	10^5	10^6	10^7	10^7	10^5	10^5	10^7	10^7
Acetic acid	0.2	3.6	10^6	10^6	10^4	10^1	10^5	10^3	<1	<1	10^5	10^5	10^5	10^5
Sorbic acid	0.1	3.8	10^6	10^3	<1	<1	10^5	10^2	<1	<1	10^5	10^5	10^3	10^1
Hydrochloric acid	0.001	3.8	10^6	10^6	10^7	10^8	10^5	10^5	10^6	10^7	10^5	10^5	10^6	10^7
Potassium sorbate	0.1	7.1	10^6	10^6	10^7	10^8	10^5	10^5	10^7	10^7	10^5	10^5	10^6	10^7
Propionic acid	0.1													
+ propionaldehyde	0.1	3.7	10^6	10^5	10^4	10^3	10^5	10^2	<1	<1	10^5	10^5	10^5	10^3
Zephirin chloride	0.006	7.2	10^6	<1	<1	<1	10^5	<1	<1	<1	10^5	<1	<1	<1
Chlorine dioxide	0.01	7.4	10^6	<1	<1	<1	10^5	<1	<1	<1	10^5	<1	<1	<1
Calcium hypochlorite	0.002[d]	7.5	10^6	<1	<1	<1	10^5	<1	<1	<1	10^5	<1	<1	<1
Iodophor	0.06[e]	3.3	10^6	<1	<1	<1	10^5	<1	<1	<1	10^5	10^1	<1	<1
Diethylpyrocarbonate	0.05	4.7	10^6	<1	<1	<1	10^5	<1	<1	<1	10^5	<1	<1	<1
Diethylpyrocarbonate	0.05													
+ acetic acid	0.1	3.8	10^6	<1	<1	<1	10^5	<1	<1	<1	10^5	<1	<1	<1
Hexylresorcinol	0.01	7.2	10^6	10^6	10^7	10^7	10^5	10^6	10^7	10^7	10^5	<1	<1	<1
Hexylresorcinol	0.005													
+ acetic acid	0.1	3.7	10^6	<1	<1	<1	10^5	<1	<1	<1	10^5	<1	<1	<1
Sodium metabisulfite	0.2	3.8	10^6	10^4	<1	<1	10^5	10^2	<1	<1	10^5	10^4	10^1	<1

[a] 1/100 dilution
[b] Counts rounded to nearest log
[c] pH of sanitizer solution
[d] Equivalent to 10 ppm available chlorine
[e] Equivalent to 10 ppm available iodine

Effective sanitizers, concentrations, and exposure times established in the pure culture study, Table 2, were applied in pilot-plant tests with RO/UF equipment. The sanitizers were held in the unit for a prescribed time, and were drained and plated immediately. This procedure obviously did not lend itself to periodic sampling as with the pure culture study, but subsequent tests showed essentially a 100% correlation of effective times and concentrations in both pure culture study and in the equipment. Data from the pure culture study are presented to show the importance of exposure time in obtaining sterility.

Organic acids, used in sufficient strength to lower pH to below 4.0, generally were effective against bacteria but not against yeasts and molds. The bactericidal properties of the acids were not entirely due to pH, as shown by the lack of control with hydrochloric acid at pH 3.8.

The chlorine compounds, calcium hypochlorite and chlorine dioxide (Dearcide - W. R. Grace and Co., Chicago, Ill.), were effective against all test organisms in a short time. Unfortunately, chlorine has been shown to be harmful to cellulose acetate membranes in concentrations as low as 50 ppm *(4)*. Since chlorine dioxide was found to be ineffective at <100 ppm, its use at that level appears undesirable except for short periods. Calcium hypochlorite gave good control at an acceptable level of 10 ppm, but it has the disadvantage of a strong flavor and odor which is difficult to remove from the modules. The chlorines share a further disadvantage in that they are most stable under alkaline conditions. This is not compatible with cellulose acetate which requires an optimum pH of 4 to 5, since the hydrolysis rate of the cellulose acetate increases rapidly as the pH approaches pH 7.0 *(11)*. The chlorines generally assumed the pH of the water used for the dilutions; thus, when alkaline water prevails, acidification would be desirable. Similarly, zephirin chloride, a quaternary ammonium compound, assumed the pH of the dilution water. Although good results were obtained with a 0.006% concentration, continued exposure to the higher pH would destroy the permeability of the membrane.

Hexylresorcinol is of interest in that it was particularly active against yeasts and molds, but not against bacteria. It had an undesirable pH, but acidification with acetic acid increased its bactericidal properties, making the combination a very effective one. Hexylresorcinol is expensive, however, and is not recommended except to cope with special problems.

Diethylpyrocarbonate (DEPC) (Baycovin-Naftone, Inc., New York, N. Y.), iodophor (Supersan-Lazarus Laboratories, Long Island, N. Y.), and sodium metabisulfite were very effective against all test organ-

TABLE 3. REJECTION OF SANITIZING AGENTS BY MEMBRANE[a]

Sanitizing agent	Original dilution		Concentrate side		Permeate side	
	pH	ppm	pH	ppm	pH	ppm
Iodophor	3.1	300	3.2	320	3.0	200
Zephirin	6.1	60	6.0	70	5.7	45
Na bisulfite	3.6	1100	3.7	1700	3.6	850

[a]Calgon-Havens Systems type 3A, NaCl rejection rate of 75%, 200 psi feed pressure.

isms in a short time. DEPC has a pleasant apple-like odor and deteriorates rapidly into harmless substances. Its main disadvantage is difficulty of use. It is insoluble in water and must first be dissolved in alcohol and care must be exercised in its handling and storage. Sodium metabisulfite, commonly used in the wine industry as an additive to stop fermentation, is very cheap and relatively odor-free. Although killing was not as rapid as with some of the others, sodium metabisulfite can be recommended for overnight shut-down when rapid sterilization is not required.

The iodophors, which have been used for years as sanitizers for dairy equipment and utensils, have considerable merit. A concentration of 24 ppm of available iodine, the maximum permitted by Federal standards, was sufficient to sterilize the unit in less than 4 hr. Excellent control was obtained at a concentration of 10 ppm. The brand of iodophor used for this study contained both glycolic and phosphoric acids, undoubtedly contributing to its overall effectiveness. Iodine has a possible disadvantage, from an esthetic standpoint in that prolonged use will probably result in staining of plastic and other non-metal components of the system.

The key requirements for adequate sanitation of the complete RO/UF unit include adaptability to design of the equipment and accessibility to all exposed surfaces on both sides of the membrane. Several designs of equipment are available and most, like the tubular type, have a shroud or other closed means of collecting the permeate. Obviously the sanitizing agent must contact both the concentrate and permeate sides of the membrane to be effective. All of the sanitizers listed in Table 2 readily passed through the porous UF membrane but rejection of some sanitizers by the intermediate RO membrane was experienced as shown in Table 3. Comparisons of the pH and concentration of dissolved solids of the diluted sanitizing solutions and the permeate show a reduction in strength of the sanitizing agent on the permeate side. Thus, it is certain that tighter RO membranes in the order of 95% NaCl rejection would hold back most of the sanitizer, resulting in little or no kill at the permeate side. This problem can be overcome by providing for a method of flooding the permeate side without going through the membrane. Flushing through the permeate manifold system would be acceptable, but both sides should be flushed simultaneously since excessive back pressure could push the membrane from its support. Systems without shrouds would require spray bars or some other method of fogging with sanitizer.

Related to this problem is the requirement of accessibility to all parts of the equipment. Currently, most tubular units are mounted horizontally. When shut off, the fluid level in the tubes and shroud drops slightly, leaving a dead area along the top for the entire length of the tubes. This dead area appears to offer ideal protection and conditions for microbial growth. Results of comparative trials in both the horizontal and vertical positions are shown in Table 4. Total counts shown for the sanitizers (a) represent the number of microbes present in the unit after 24 hr contact with the sanitizer. The sanitizer was then flushed out of the unit with sterile tap water. Counts of the flush water (b) represent growth that occurred during the next 24 hr. When mounted vertically, both the concentrate and permeate outlets were at the top, thus assuring complete flooding. Results shown are typical, but in some instances, control in the horizontal position was as good as in the vertical position. Thus, it is evident that, while good

TABLE 4. EFFECT OF MODULE POSITION ON EASE OF SANITATION

Sanitizer	%	Total counts/ml			
		Vertical position		Horizontal position	
		Concentrate side	Permeate side	Concentrate side	Permeate side
Iodophor[a]	0.06	0	0	0	12
Subsequent flush water[b]	—	0	0	0	20×10^4
Sodium metabisulfite[a]	0.15	2	0	5	40×10^2
Subsequent flush water[b]	—	40	0	25×10^2	68×10^3

[a]After 24 hr contact with sanitizer
[b]Sterile water used to flush sanitizer from unit and held for 24 hr.

control is possible, sterilization cannot be assured when the modules are in the horizontal position. It should be emphasized that this may apply only to equipment tested here; other manufacturers' equipment, though tubular, may differ sufficiently in design to eliminate the problem.

Choice of sanitizer should take into account all of the factors discussed here, and would naturally depend on the degree of control desired, the particular operation of the plant, and special problems encountered. Obviously, a plant operating on a 10 hr day would have more latitude than one operating on a 20 hr day.

Since the presence of large amounts of organic matter in the equipment would seriously affect the efficacy of any sanitizer, some mention of a cleaning procedure is desirable. Cleaning problems will naturally depend on the unit design and the composition and nature of the feed material. Experience has shown that some accumulation of material at the membrane surface may contribute to clogging. Lim, et al. have identified the build-up as a gel-like layer of casein and whey proteins *(5)*. Since these deposits resist removal by flushing, preliminary flushing with an enzyme active detergent may be necessary. Approximately 0.1 g of detergent and 0.5 g of sodium hexametaphosphate should be added to each liter of water. The cleaning solution should be adjusted to pH 7.0 or lower, and should be flushed through the unit at 40 to 50 F to prevent excessive permeation. After 30 to 40 min, the solution should be flushed from the unit and replaced with a suitable sanitizing solution.

Our experience has shown that when RO/UF equipment is adequately cleaned, good microbial control can be maintained using any of the several effective agents shown in Table 2. However, based on effectiveness, economy, ease of handling, and ease of removal from the modules, the iodophors appear to be the most logical choice for general use.

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